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Insecticide Persistence in Natural Seawater as Affected by Salinity, Temperature, and Sterility

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No significant differences between sterile and nonsterile treatments were observed for any of the insecticides studies, while the effect of increasing temperature was highly significant with regard to increased degradation of malathion, parathion, methyl parathion, and diazinon. Methoxychlor reflected the recalcitrance characteristic of the chlorinated hydrocarbon insecticides throughout 84 days of incubation and was not significantly affected by salinity, temperature, or sterility. Salinity effects were varied among the four organophosphates, being highly significant for malathion and diazinon, significant for methyl parathion, and not significant for parathion.

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INSECTICIDE PERSISTENCE IN NATURAL SEAWATER AS AFFECTED BY SALINITY, TEMPERATURE, AND STERILITY

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FOREWORD

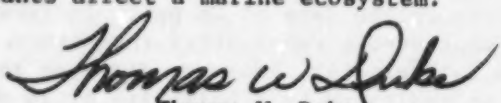
The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

the effects of toxic organic pollutants on individual species and communities of organisms;

the effects of toxic organics on ecosystem processes and components;

the significance of chemical carcinogens in the estuarine and marine environments.

Due to the increased use of biodegradable pesticides, it becomes imperative that the fate of these compounds be determined to properly assess their impact on the environment. Information on the effects of physical, chemical, and biological factors on toxic organics in estuarine waters, as produced by this report, will contribute greatly to the scientific assessment of how biodegradable pollutants affect a marine ecosystem.



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ABSTRACT

The effect of temperature, salinity, and sterility on the degradation of malathion, parathion, methyl parathion, diazinon, and methoxychlor in fresh and estuarine water has been determined under controlled laboratory conditions. Surface water samples of 0, 10, 20, and 28 ppt salinity were amended with the above insecticides and incubated in the dark at 30, 20, and 10°C under sterile and nonsterile conditions. Insecticide abatement was followed by electron-capture gas-liquid chromatographic techniques.

No significant differences between sterile and nonsterile treatments were observed for any of the insecticides studied, while the effect of increasing temperature was highly significant with regard to increased degradation of malathion, parathion, methyl parathion, and diazinon. Methoxychlor reflected the recalcitrance characteristic of the chlorinated hydrocarbon insecticides throughout 84 days of incubation and was not significantly affected by salinity, temperature, or sterility. Salinity effects were varied among the four organophosphates, being highly significant for malathion and diazinon, significant for methyl parathion, and not significant for parathion.

Malathion was the shortest-lived of the insecticides tested, with half-lives at 30°C varying from approximately 11 days in fresh water to less than two days at 10, 20, or 28 ppt salinity. The rate of methyl parathion disappearance was second only to malathion and ranged, in terms of half-lives, from 27 days in fresh water to 16 days at 28 ppt. In fresh water, a 45-day half-life for diazinon suggested a substantial resistance to degradation, especially at 30°C. In saline water, however, diazinon abatement was considerably accelerated, as indicated by a half-life of 24 days at 28 ppt salinity. Parathion, the most persistent of the organophosphate insecticides tested, reflected a half-life of at least 44 days regardless of salinity.

One bacterium, tentatively identified as Moraxella sp., was isolated from sediment by enrichment and proved capable of readily degrading malathion either as a primary carbon source or in the presence of peptone. Of two bacteria tested for the ability to degrade methyl parathion, one, possibly a Pseudomonas sp. proved quite capable of utilizing the insecticide with or without peptone, while the other, a Moraxella species, reflected no degradation of methyl parathion as the primary carbon source and only limited utilization in the presence of peptone. Neither of two bacteria screened for parathion metabolism were capable of insecticide degradation under the conditions of this evaluation.

This report was submitted in fulfillment of Grant No. R-803842 by the Gulf Coast Research Laboratory, Ocean Springs, MS, under sponsorship of the U.S. Environmental Protection Agency. This report covers the period July 15, 1975 through July 14, 1977, and work was completed September 30, 1977.

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SECTION 1

INTRODUCTION

The organophosphorus group of insecticide chemicals currently enjoys wide usage throughout the United States, due in part to various bans and restrictions on the so-called "hard" chlorinated hydrocarbon and related pesticides. And, while the organophosphorus insecticides are as a whole considered relatively safe, certain individual members of the group are potent acetylcholinesterase inhibitors (20), and their role as potential environmental hazards cannot be overlooked.

Considerable information is available regarding the overall fate of organophosphorus insecticides in soil (6, 7, 12, 16, 18, 19, 22, 23), their degradation in terrestrial plants and animals (1, 5, 15), and their toxicities and effects on freshwater species (4, 8, 9, 12, 17). Comparatively little information, however, is available concerning the disposition of the organophosphorus insecticides in the estuarine or salt-marsh environment (2, 10, 11, 20). This deficit, coupled with the fact that the use of organophosphorus insecticides in and around estuarine areas is increasing due to various mosquito control programs (3), the research effort described herein was formulated.

The overall objective of these investigations was (1) to determine the effect of temperature, salinity, and sterility on the persistence and degradation of representative organophosphorus and chlorinated hydrocarbon insecticides in natural seawater, and (2) to isolate into pure culture representative estuarine microorganisms capable of utilizing these materials either as a primary carbon source or in the presence of an additional energy-rich material.

SECTION 2

CONCLUSIONS

It is concluded from this investigation that the effect of increasing temperature is highly significant with regard to the breakdown of malathion, parathion, methyl parathion, and diazinon in natural water throughout the zero tc 28 ppt salinity range of this study. Although not demonstrated by actual data, this relationship probably exists for the organophosphorus insecticide group as a whole. The significance of observed salinity effects on insecticide abatement (99% for malathion and diazinon, 95% for methyl parathion, 42% for parathion), on the other hand, appears to vary from compound to compound with little uniformity within the group. Sterility effects appear minimal, as do treatment interactions. The recalcitrance of methoxychlor observed under all conditions of this investigation seems to reaffirm the persistent nature of the chlorinated hydrocarbon family of insecticides over a wide range of environmental conditions.

Of the three compounds tested, malathion and methyl parathion were degraded microbiologically either as primary carbon sources or in the presence of an additional energy supply, while parathion remained immune to breakdown under either of these conditions. This information indicates that while the organophosphate insecticides may be generally considered non-persistent, particular numbers of the group may well exhibit a substantial degree of resistance to chemical or microbial attack.

SECTION 3

RECOMMENDATIONS

It is recommended that as the use of organophosphorus, carbamate, and related "short-lived" pesticides increases, a concomitant broadening of knowledge regarding the fate and effect of these materials in the natural environment be observed. Investigation along these lines should embrace chemical as well as biological mechanisms, probably through the microcosm and simulated ecosystem approach.

SECTION 4

MATERIALS AND METHODS

WATER SAMPLES

Surface water samples of 0, 10, 20, and 28 mg/ml (ppt) salinity were collected as required from the Biloxi Bay - Mississippi Sound estuary system located geographically near Biloxi and Ocean Springs, Mississippi. Samples were collected on a transect originating in Fort Bayou, traveling through Biloxi Bay and into the Mississippi Sound, and terminating approximately one (1) mile due south of the west end of Horn Island. Water samples were collected by dipping chemically clean 3.79 liter (one gallon) amber glass containers fitted with Teflon® lined caps.

Water samples were returned to our laboratory, filtered through glass wool, and dispensed in 25 ml aliquots into 0.13 liter (8 oz.) prescription bottles. Sterilization, where appropriate, was accomplished by autoclaving for 15 minutes at 121°C and 6.8 kg (15 pounds) pressure, and representative autoclaved treatments were plated on half-strength marine agar to confirm sterility.

INSECTICIDES AND INCUBATION PARAMETERS

Insecticides employed in these investigations were malathion (S-[1,2-Dicarbethoxyethyl]-O,O-dimethyldithiophosphate), parathion (O,O-Diethyl-O,p-nitrophenyl phosphorothioate), methyl parathion (O,O-Dimethyl-O,p-nitrophenyl phosphorothioate), diazinon (O,O-Diethyl-O-[2-isopropyl-4-methyl-6-pyrimidinyl] phosphorothioate), and methoxychlor (1,1,1-Trichloro-2,2-bis[p-methoxyphenyl] ethanol).

Insecticides were filter-sterilized using standard Millipore filtration apparatii fitted with 0.4 micron Nuclepore filter pads and added to respective incubation vessels as 0.5 ml acetone solutions. Insecticide concentrations employed in these investigations were: malathion, 5.04 mg/l; parathion, 4.48 mg/l; diazinon, 1.81 mg/l; methyl parathion, 6.96 mg/l; and methoxychlor, 1.04 mg/l. All incubations were conducted in the dark at 10°, 20°, and 30°C in Freas model 815 incubators.

INSECTICIDE EXTRACTION AND ANALYSIS

Following incubation, residual insecticides were extracted with 100 ml of a 1:1 (v:v) ratio of hexane and acetone, both pesticidequality solvents. Diazinon (90.62 µg) was added to malathion, parathion, and methyl parathion incubations as an internal standard immediately prior to extraction. Parathion (112.06 µg) was added as internal standard for diazinon incubations, and heptachlor (1,4,5,6,7,8,8-Heptachloro-3a.4.7.7a-tetrahydro-4,7-methanoindene, 30.04 µg) was used as the internal standard for methoxychlor incubations. Acetone was subsequently removed by washing with distilled water, and final traces of water were removed by passage through anhydrous sodium sulfate. The dried hexane extract was then analyzed for residual insecticides, using two Tracor MT-220 gas chromatographs, each equipped with one Ni^{63} electron-capture and one flame photometric detector. Glass columns, 0.6 cm (0.25 in.) inside diameter, and varying in length from 0.9 to 1.8 m (3 to 6 ft.) and two column packings, 1.5% OV-17 + 1.95% QF-1 and 6% QF-1 + 4% SE-30, both on chromosorb W, HP, 100/120 mesh, were used throughout these investigations. Nitrogen served as carrier and purge gas at flow rates of 95 and 20 ml/min, respectively. Operating temperatures were as follows: inlet, 200°C; column oven, 185°C; electron-capture detectors, 275°C; and flame photometric detectors (ignited), 215°C.

MICROBIAL DEGRADATION STUDIES

Estuarine microorganisms capable of degrading malathion, parathion, methyl parathion, and diazinon were sought by enrichment. Approximately 3 cc of surface sediment was collected from Davis Bayou, an area of the Mississippi Sound immediately adjacent to our laboratory, and diluted to 100 ml with artificial seawater of 15 ppt salinity. Each sediment suspension was then amended with 7312 µg malathion, 11,206 µg parathion, or 8697 µg methyl parathion at two-day intervals for a period of 22 days. Temperature of incubation during enrichment was 30°C in all cases. Following enrichment, all treatments were plated onto half-strength marine agar, and all different colonies isolated into pure culture. The predominant isolates in each case were then tested for the ability to utilize its enrichment insecticide both as the primary carbon source (insecticide added in 0.5 ml acetone) or in the presence of 1% peptone (Bacto-Peptone, Difco Laboratories, Cat. No. 011801), a readily available energy source.

Each isolate tested was grown in mass on half-strength marine agar bottle slants, washed three times with physiological saline (0.85% sodium chloride in distilled water) to remove "carry-over" nutrients, and added to 25 ml mineral salts solution (13) containing either insecticide alone or insecticide plus 1% peptone. All treatments were incubated in the dark at 25°C. Respective malathion, parathion, and methyl parathion concentrations in these investigations were 5.86, 4.46, and 6.20 mg/l.

SECTION 5

RESULTS AND DISCUSSION

INSECTICIDE LOSS FROM NATURAL SEAWATER

Malathion

Losses of malathion from natural seawater as affected by temperature, salinity, and sterility are illustrated in Table A-1 and Figure 1. It should be noted at this point that analysis of variance and chi-square analyses did not indica e that sterility was a significant factor in the disappearance of malathion from natural seawater. Both sterile and nonsterile data are listed in Table A-1, but only nonsterile results are shown in Figure 1. At 30°C (Figure 1, top), malathion abatement was quite rapid and in direct proportion to increasing salinity. At 20 and 28 ppt, malathion could not be detected after 10 days incubation. At 10 ppt, all of the added malathion had disappeared after 14 days. In fresh water, malathion was more persistent, with losses ranging from 30 percent at three days to 63 percent after 20 days.

At 20°C (Figure 1, center), malathion abatement was similar to that observed at 30°C except that the rate of disappearance was reduced. At 28 ppt salinity, malathion degradation was complete after 15 days incubation as compared to 20 days at 20 ppt and 25 days at 10 ppt. Malathion disappearance was again slowest in fresh water (0 ppt), with losses ranging from 22 percent after one day to 67 percent after forty days.

Incubation at 10°C greatly reduced the rate of malathion breakdown, as indicated in Figure 1, bottom. At 28 ppt, insecticide losses ranged from 34 percent at four days to 97 percent after 70 days. At 20 ppt, malathion disappearance ranged from 27 to 94 percent over the same period of time as compared to a range of 26 to 89 percent in the 10 ppt incubations. At 0 ppt, malathion abatement was extremely slow, with losses ranging from eight percent at four days to only 27 percent at the end of the 70-day incubation period.

Generally speaking, malathion abatement was rapid under all incubation conditions except zero salinity and 10°C. Analysis of variance revealed that both temperature and salinity effects were significant at the 99% level of probability, while sterility reflected a significance of only 72.8 percent. Levels of significance for a temperature/salinity interaction, a temperature/sterility interaction, or a salinity/sterility interaction were 81.5, 29.6, and 13.6 percent, respectively, revealing that no significant (at the 95% level of probability) two-way interactions occurred. The three-factor level of significance was only 8.6 percent.

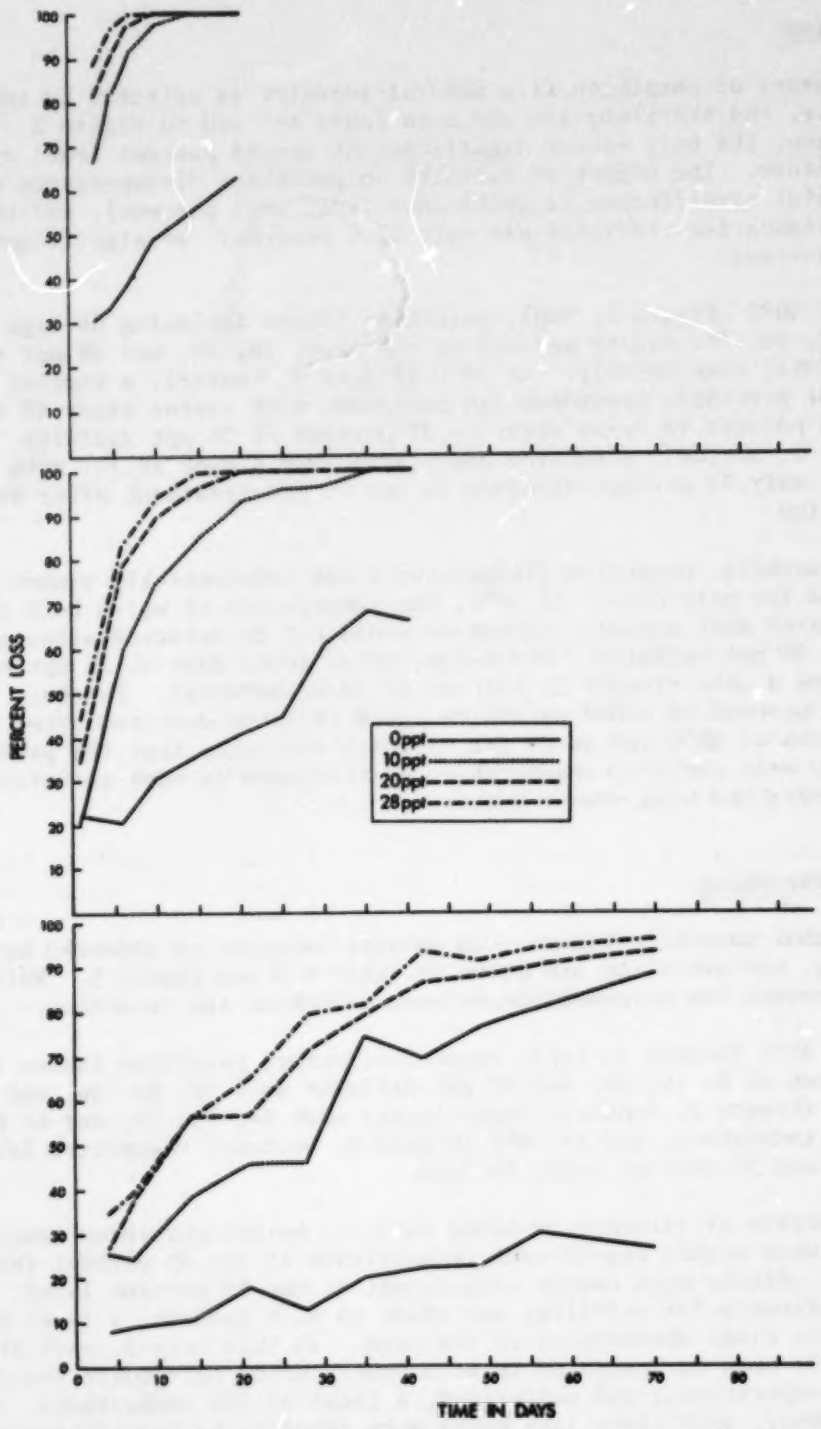

Figure 1. Malathion disappearance from nonsterile water at 0, 10, 20, and 28 ppt salinity and temperatures of 30° (top), 20° (center), and 10°C (bottom).

Parathion

Losses of parathion from natural seawater as affected by temperature, salinity, and sterility are shown in Table A-2 and in Figure 2. Unlike malathion, the only effect significant at the 99 percent level was temperature. The effect of salinity on parathion disappearance was of no meaningful significance (significance level 46.1 percent), and the level of significance for sterility was only 22.9 percent. No significant interactions were observed.

At 30°C (Figure 2, top), parathion losses following 84 days incubation were 78, 76, 78, and 68 percent in the zero, 10, 20, and 28 ppt salinity treatments, respectively. At 20°C (Figure 2, center), a similar but retarded trend of parathion breakdown was observed, with losses after 84 days ranging from 23 percent in fresh water to 37 percent at 28 ppt salinity. At 10°C (Figure 2, bottom), parathion degradation was slower still, with a maximum loss of only 27 percent observed in the 28 ppt treatment after 84 days incubation.

Generally, parathion disappearance was substantially slower than that observed for malathion. At 30°C, the temperature at which both insecticides disappeared most rapidly, malathion could not be detected after only seven days at 28 ppt salinity. Parathion, after seven days at 28 ppt salinity, reflected a loss of only 11 percent of added material. Further, the fact that some 32 percent of added parathion could still be detected after 84 days incubation at 30°C and at 28 ppt salinity indicates that the parathion molecule may well exhibit considerable recalcitrance in high-salinity seawater even during the warm summer months.

Methyl Parathion

Methyl parathion losses from natural seawater as affected by temperature, salinity, and sterility are shown in Table A-3 and Figure 3. Methyl parathion disappearance was intermediate between malathion and parathion.

At 30°C (Figure 3, top), respective methyl parathion losses after 43 days incubation at 0, 10, 20, and 28 ppt salinity were 82, 82, 90, and 83 percent. At 20°C (Figure 3, center), these losses were 64, 63, 75, and 67 percent after 90 days incubation, and at 10°C (Figure 3, bottom), respective losses were 25, 25, 31, and 31 percent after 90 days.

Analysis of variance revealed that for methyl parathion temperature effects were highly significant (significant at the 99 percent level), while salinity effects were barely significant at the 95 percent level. The level of significance for sterility was shown to 97.6 percent, a level that appeared high after close observation of the data. In this regard, each sterile and nonsterile mean was compared to each other, using Chi-Square analyses for all times, temperatures, and salinities, a total of 200 comparisons. Of this total number, only three loss means were found to be significantly different

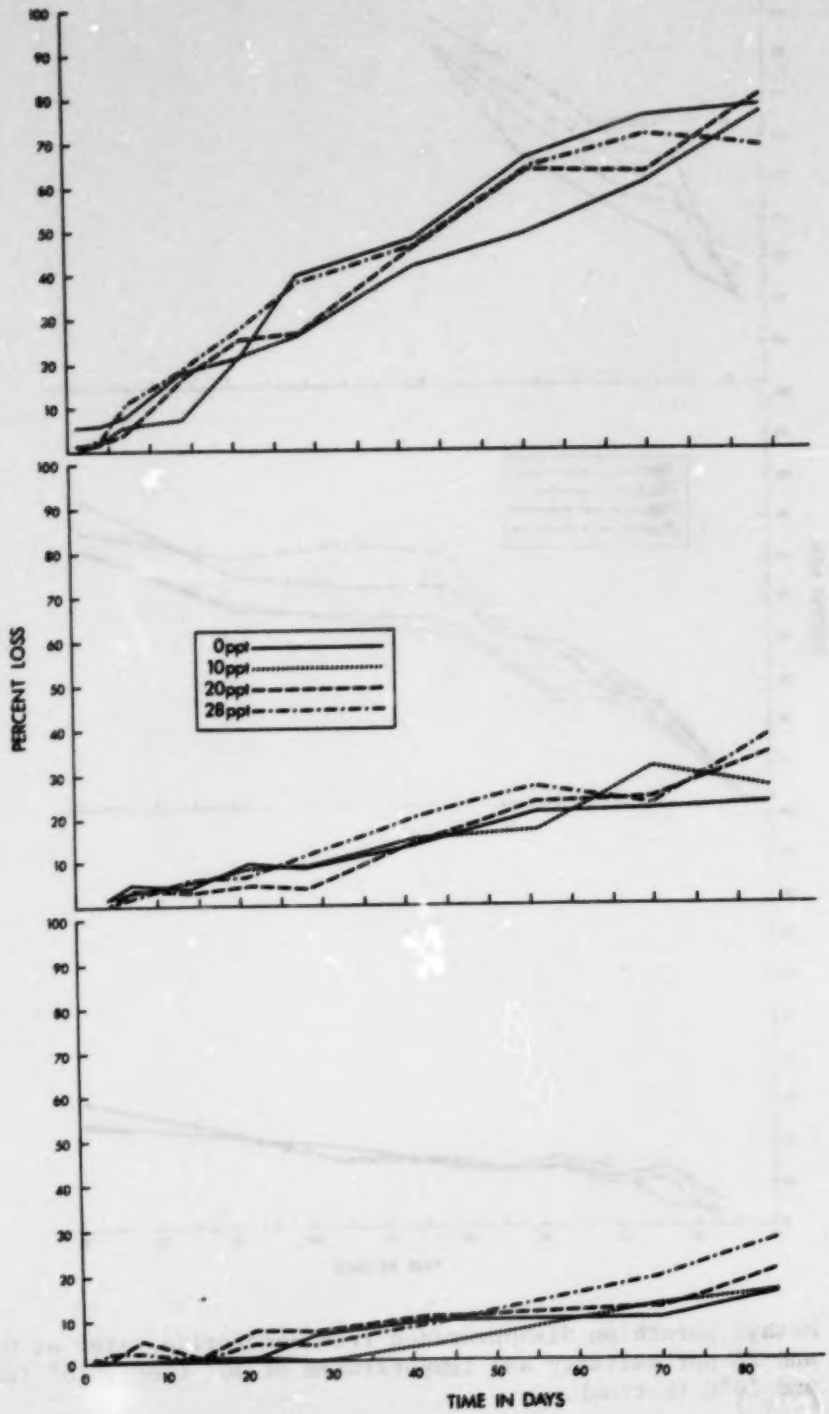

Figure 2. Parathion disappearance from nonsterile water at 0, 10, 20, and 28 ppt salinity and temperatures of 30° (top), 20° (center), and 10°C (bottom).

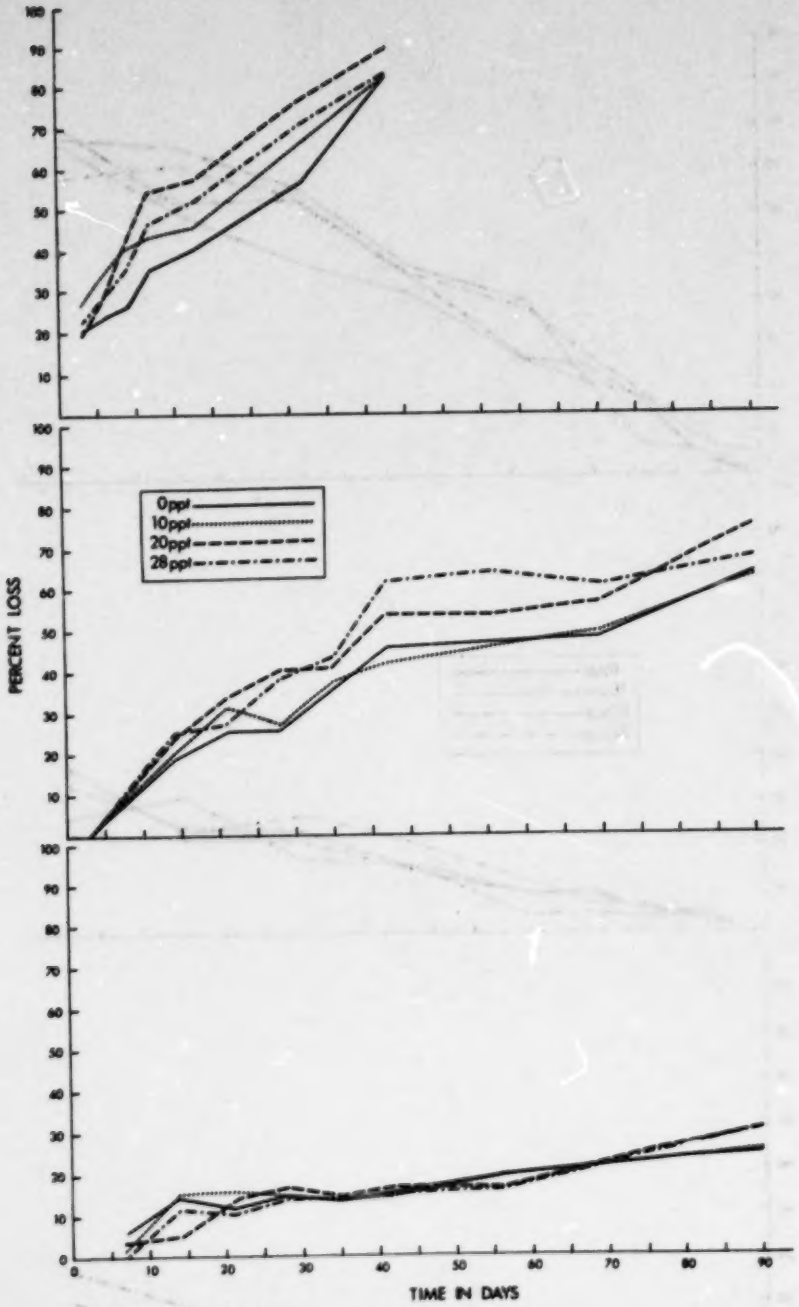

Figure 3. Methyl parathion disappearance from nonsterile water at 0, 10, and 28 ppt salinity and temperatures of 30° (top), 20° (center), and 10°C (bottom).

due to sterility at the 95 percent confidence level, indicating that, in reality, sterility is of no statistical consequence regarding the disappearance of methyl parathion from seawater. No significant treatment interactions were observed for methyl parathion.

Diazinon

Diazinon losses from natural seawater as affected by temperature, salinity, and sterility are shown in Table A-4 and Figure 4. At 30°C (Figure 4, top), diazinon disappearance was intermediate between parathion and methyl parathion. Losses ranged from 70 percent in fresh water to 80 percent at 10 ppt salinity to 81 percent at 20 ppt to 87 percent at the highest salinity of 28 ppt. Diazinon dissipation was similar to that observed with malathion in that the effect of salinity was significant at the 99 percent confidence level. The rate of breakdown, however, was greatly reduced. At 20°C (Figure 4, center), respective diazinon losses for 0, 10, 20, and 28 ppt salinity were 33, 40, 62, and 61 percent, as compared to 15, 18, 24, and 21 percent at 10°C (Figure 4, bottom). As was the case with malathion, parathion, and methyl parathion, the effect of temperature on the degradation of diazinon was significant at the 99 percent level. No significant interactions were observed.

Methoxychlor

Methoxychlor losses from natural seawater as affected by temperature, salinity, and sterility are shown in Table A-5. At 30°C, methoxychlor losses after 84 days ranged from 12 percent in fresh water to nine percent at 28 ppt. Methoxychlor losses after 84 days at 20°C were in the five to eight percent range, and losses at 10°C were less than two percent throughout the 84-day incubation period. The recalcitrance displayed by methoxychlor under all test conditions in these investigations is characteristic of the chlorinated hydrocarbon insecticide group in general and clearly delineates the hazard these materials represent as environmental pollutants.

Insecticide Half-Life

Figure 5 illustrates the times required to achieve 50 percent loss of each of the four organophosphorus insecticides employed in these studies when incubated in the dark at 30°C and at 0, 10, 20, and 28 ppt salinity. These times were taken from Figures 1-4 by interpolation and hence are approximate in most cases and intended solely for use in comparing the four insecticides to one another. The height of the bars in Figure 5 is indicative of the time in days, and the numbers above the bars represent the salinity in parts per thousand.

The half-life of malathion in fresh water is approximately 11 days and is the shortest of the four. Malathion dissipation is greatly enhanced in saline water, as evidenced by the drop in half-life to less than two days in 10, 20, and 28 ppt salinity water.

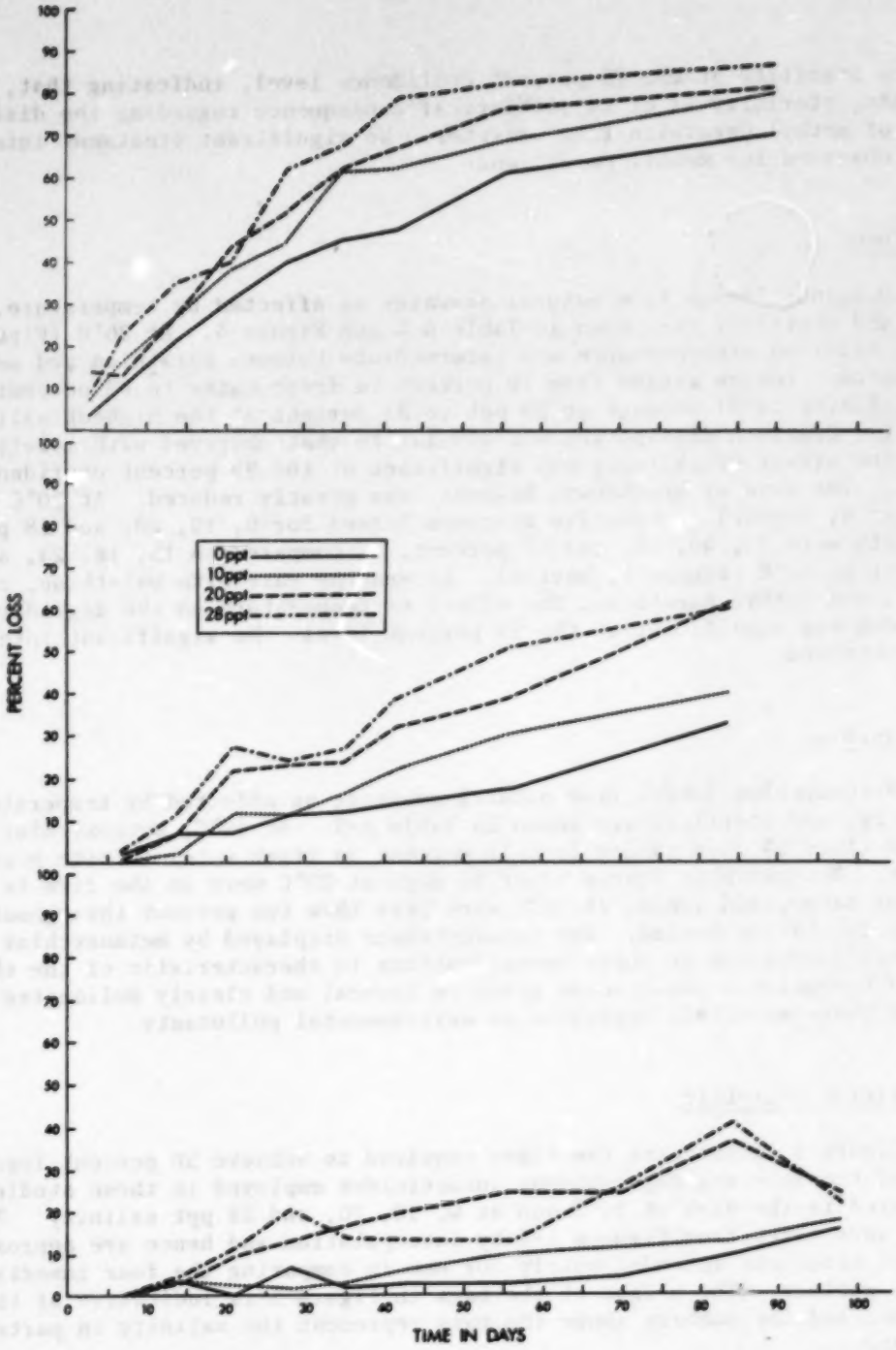

Figure 4. Diazinon disappearance from nonsterile water at 0, 10, 20, and 28 ppt salinity and temperatures of 30° (top), 20° (center), and 10°C (bottom).

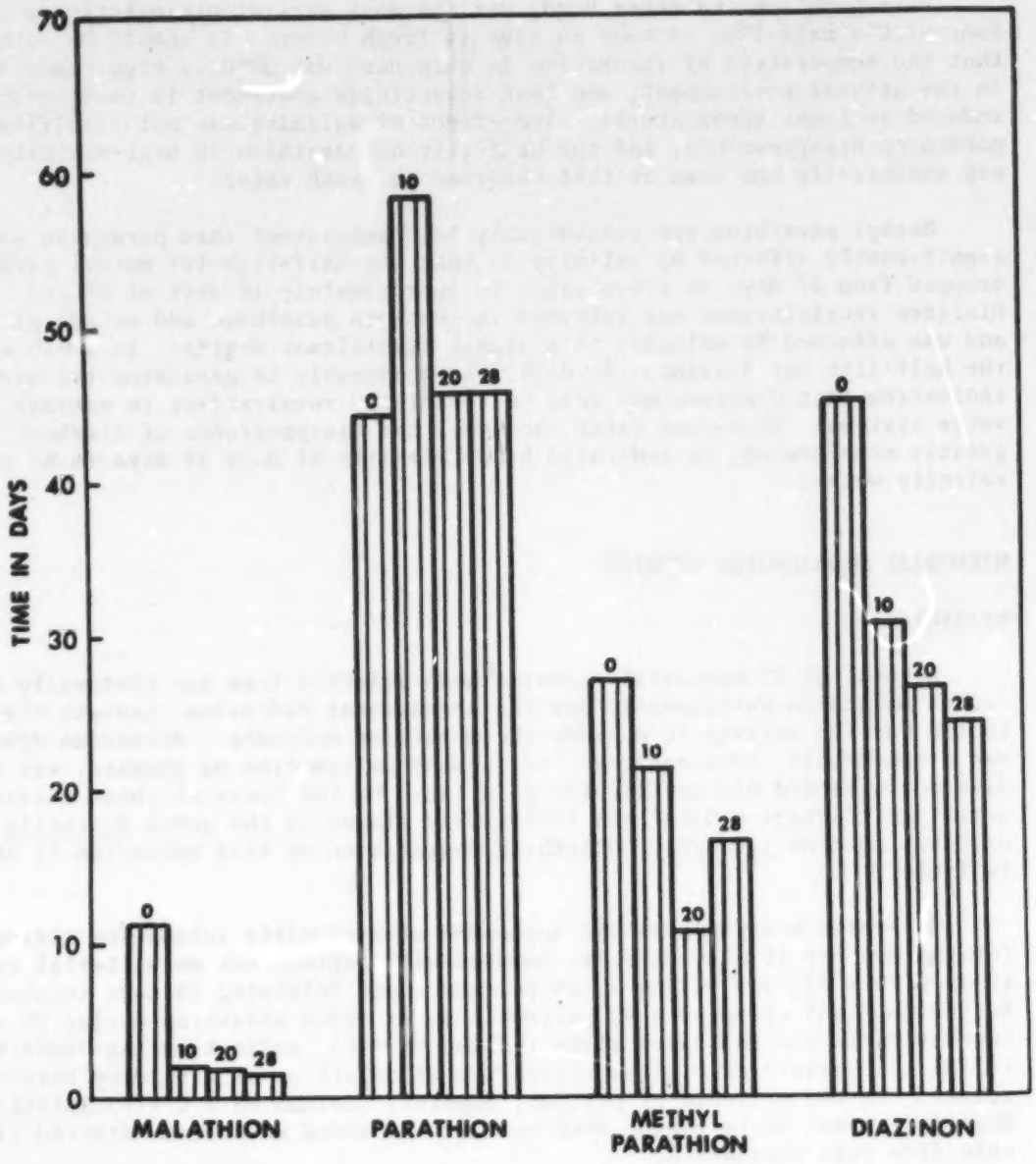

Figure 5. Insecticide half-life at 30°C and 0, 10, 20 and 28 ppt salinity.

Parathion, on the other hand, was the most persistent insecticide of the four with a half-life of some 44 days in fresh water. It should be noted here that the temperature of incubation in this case was 30°C, a high temperature in the natural environment, and that insecticide abatement is considerably reduced at lower temperatures. The effect of salinity was not significant in parathion disappearance, and the half-life of parathion in high-salinity water was essentially the same as that observed in fresh water.

Methyl parathion was considerably less persistent than parathion and was significantly affected by salinity in that the half-life for methyl parathion dropped from 27 days in fresh water to approximately 16 days at 28 ppt. Diazinon recalcitrance was intermediate between parathion and methyl parathion and was affected by salinity to a highly significant degree. In fresh water tne half-life for diazinon (45 days) was comparable to parathion (44 days), indicating that diazinon may well be relatively recalcitrant in natural fresh water systems. In saline water, however, the disappearance of diazinon is greatly accelerated, as indicated by a half-life of only 24 days in 28 ppt salinity water.

MICROBIAL DEGRADATION STUDIES

Malathion

A total of 25 bacterial colonies were selected from the previously described malathion enrichments, and the predominant bacterium, isolate M14A1, tested for the ability to degrade the malathion molecule. Bacterium M14A1 was a non-motile, Gram negative rod, showed no reaction on glucose, was oxidase positive and did not liquify gelation. On the basis of these characteristics, bacterium M14A1 was tentatively placed in the genus Moraxella, probably species locunata. Malathion degradation by this bacterium is shown in Table 1.

Malathion breakdown in the unamended mineral salts incubation medium (Column MS), or in mineral salts amended with peptone but no bacterial cells (Column MS + P), was in the 27-28 percent range following 28 days incubation. Bacterium M14A1 effected a 63 percent loss of added malathion during 28 days incubation in mineral salts alone (Column MS + C), indicating that this bacterium was capable of limited metabolism of malathion as a primary carbon source. In the presence of peptone, however, (Column MS + C + P) malathion degradation was quite rapid, with none of the added malathion detected after only five days incubation.

Parathion

Eight individual bacteria were isolated from the parathion enrichments, and the two predominant organisms (P14B1 and P25C2) tested for the ability to degrade parathion. Both of these bacteria reflected motility, Gram, glucose, oxidase, and gelatin reactions identical to bacterium M14A1 and were, hence, thought to be Moraxella species. Neither of the two cultures tested (Table 2) reflected any real ability to utilize the parathion molecule, either as the

primary carbon source or in the presence of peptone. Although only two of the eight bacteria available could be tested in the time available, the fact that the two bacteria that were tested represented the two most abundant species present in the parathion enrichment and could not appreciably degrade the parathion molecule certainly indicates a fair amount of resistance on the part of the parathion molecule to microbial attack.

TABLE 1. MALATHION DEGRADATION BY ISOLATE M14A1

Days incubation	Percent loss* of added insecticide			
	MS	MS + C	MS + P	MS + C + P
1	2	0	4	11
3	4	6	4	46
5	6	9	7	100
7	9	14	13	100
10	14	26	16	100
15	19	38	21	100
20	22	54	23	100
28	28	63	27	100

*The losses listed represent means of duplicate treatments. Column headings are as follows: MS, mineral salts plus insecticide; MS + C, mineral salts plus insecticide plus culture; MS + P, mineral salts plus insecticide plus 1% peptone; MS + C + P, mineral salts plus insecticide plus culture plus 1% peptone. Incubation temperature was 25 C in all cases.

Methyl Parathion

Degradation of methyl parathion by two bacteria, MP15A1 and MP25C2, is illustrated in Table 3. Bacterium MP25C1 was tentatively identified as belonging to the genus Moraxella, while isolate MP15A1, a motile, Gram negative rod was placed in the genus Pseudomonas. A total of 23 individual bacteria were isolated from the methyl parathion enrichment, but time permitted testing of only these two.

MP15A1 appeared able to utilize methyl parathion as the primary carbon source, as evidenced by a loss of 59 percent of the added methyl parathion in mineral salts plus bacterium (Column MS + C), as compared to only 21 percent loss in the unamended mineral salts. This bacterium's ability to degrade methyl parathion was substantially enhanced in the presence of energy-rich peptone, as evidenced by the fact that all of the added methyl parathion was completely degraded after 10 days incubation (Column MS + C + P). Methyl parathion abatement in the presence of peptone but no cells (Column MS + P) was not significantly different from that in the unamended mineral salts.

TABLE 2. PARATHION DEGRADATION BY ISOLATES P14B1 AND P25C2

Days incubation	Percent loss* of added insecticide			
	MS	MS + C	MS + P	MS + C + P
		P14B1		
1	1	0	0	0
3	0	0	2	3
5	3	3	1	4
7	3	2	4	6
10	5	6	4	9
15	8	10	6	13
20	10	10	9	15
28	13	12	11	22
		P25C2		
1	0	1	0	1
3	0	1	1	0
5	3	4	0	1
7	3	4	3	2
10	6	8	6	5
15	8	11	10	11
20	12	14	16	17
28	16	17	19	22

*See footnote, Table 1.

Diazinon

A total of nine bacteria were isolated from the previously described enrichment treatment, but, unfortunately, time has not permitted the testing of any of these organisms for their ability to degrade the diazinon molecule.

TABLE 3. METHYL PARATHION DEGRADATION BY ISOLATES MP15A1 AND MP25C1

Days incubation	Percent loss* of added insecticide			
	MS	MS + C	MS + P	MS + C + P
		MP15A1		
1	5	3	1	4
3	6	7	5	10
5	8	15	7	29
7	9	22	10	48
10	12	33	11	69
15	14	40	13	100
20	19	46	15	100
28	21	59	17	100
		MP25C1		
1	4	3	4	3
3	5	6	5	5
5	7	7	6	8
7	9	8	9	13
10	10	9	11	18
15	12	11	14	29
20	14	12	16	34
28	16	16	17	42

*See footnote, Table 1.

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APPENDIX

TABULATED DATA - INSECTICIDE LOSS VS. TIME

TABLE A-1. PERCENT LOSS* OF MALATHION FROM NATURAL SEAWATER AS AFFECTED BY TEMPERATURE, SALINITY, AND STERILITY

Days incubation	Temp. °C	Salinity (ppt) 0 S	0 NS	10 S	10 NS	20 S	20 NS	28 S	28 NS
3		28	30	65	66	74	80	78	88
5		35	33	77	80	88	91	89	96
7	30	44	39	93	91	92	98	96	100
10		56	49	95	97	97	100	100	100
14		53	54	98	100	100	100	100	100
20		68	63	100	100	100	100	100	100
1		18	22	21	20	35	35	45	44
6		17	21	67	59	77	79	85	84
10		19	30	75	75	90	90	94	94
15		36	36	85	85	96	97	100	100
20	20	43	40	94	93	100	100	100	100
25		51	45	100	100	100	100	100	100
30		46	60	100	100	100	100	100	100
35		50	69	100	100	100	100	100	100
40		61	67	100	100	100	100	100	100
4		7	8	20	26	23	27	28	34
7		8	9	21	26	32	38	33	40
14		--	10	29	39	44	56	54	56
21		10	18	40	46	52	57	63	65
28	10	11	13	41	47	56	72	72	80
35		22	20	62	75	75	80	81	82
42		17	23	66	69	78	87	88	94
49		23	23	69	77	81	88	88	92
56		31	31	76	81	87	91	92	95
70		28	27	82	89	88	94	94	97

*Means of duplicate treatments.

TABLE A-2. PERCENT LOSS* OF PARATHION FROM NATURAL SEAWATER AS AFFECTED BY TEMPERATURE, SALINITY, AND STERILITY

Days incubation	Temp. °C	Salinity (ppt) 0 S	0 NS	10 S	10 NS	20 S	20 NS	28 S	28 NS
1		3	6	2	0	1	0	0	0
4		7	6	1	2	3	2	3	3
7		8	8	4	5	5	4	13	11
14		13	18	21	9	16	16	24	18
21	30	18	22	23	22	25	26	34	28
28		28	39	31	26	33	27	36	38
42		39	48	42	42	44	46	44	46
56		54	66	54	50	56	63	66	63
70		56	75	60	61	60	62	70	70
84		58	78	65	76	65	78	66	68
4		0	2	2	2	2	0	2	1
7		1	5	4	4	4	4	3	2
14		3	4	4	5	8	3	4	5
21	20	8	9	7	9	10	4	6	7
28		7	9	11	9	8	4	11	11
42		12	14	16	16	29	15	20	20
56		20	21	22	17	40	23	26	27
70		19	22	22	31	48	24	31	22
84		31	23	31	27	51	33	47	37
1		4	0	0	0	0	1	0	0
3		4	0	2	0	0	0	3	3
7		1	0	2	0	6	5	2	3
14		4	1	1	0	7	0	0	1
21	10	6	1	4	1	3	7	2	5
28		8	6	6	0	2	7	3	4
42		6	9	8	4	10	10	9	8
70		14	9	12	13	17	12	15	19
84		21	15	14	16	17	20	18	27

*Means of duplicate treatments.

TABLE A-3. PERCENT LOSS* OF METHYL PARATHION FROM NATURAL SEAWATER AS AFFECTED BY TEMPERATURE, SALINITY, AND STERILITY

Days incubation	Temp. °C	Salinity (ppt) 0 S	0 NS	10 S	10 NS	20 S	20 NS	28 S	28 NS
3		19	20	18	27	16	19	18	22
6		23	24	23	34	23	29	24	29
9		22	26	31	40	33	42	33	36
12	30	30	35	34	43	40	55	40	46
18		33	40	36	46	40	58	47	52
32		41	56	51	66	60	77	64	71
43		76	82	80	82	82	90	82	83
3		0	0	0	0	0	0	0	0
14		22	19	21	20	23	23	19	25
21		24	26	30	42	24	32	24	27
28		26	26	27	22	33	40	31	38
35	20	33	36	36	39	40	41	38	43
42		40	45	43	42	48	54	46	52
56		35	44	40	45	43	53	48	56
70		36	48	46	50	48	56	52	61
90		44	64	49	63	58	75	61	67
7		4	6	7	1	5	3	2	0
14		13	14	17	15	17	5	14	12
21		15	13	18	16	17	14	15	10
28		17	15	15	15	16	17	16	14
35	10	14	14	14	14	17	15	16	14
42		16	16	16	14	17	17	16	17
56		19	19	21	20	20	17	19	17
77		26	23	23	23	26	26	25	25
90		24	25	26	25	29	31	26	31

*Means of duplicate treatments.

TABLE A-4. PERCENT LOSS* OF DIAZINON FROM NATURAL SEAWATER AS AFFECTED BY TEMPERATURE, SALINITY, AND STERILITY

Days incubation	Temp. °C	Salinity (ppt) 0 S	0 NS	10 S	10 NS	20 S	20 NS	28 S	28 NS
3		4	4	5	7	8	14	9	9
7		10	10	13	16	15	13	24	22
14		17	22	22	27	28	27	31	35
21	30	27	31	35	38	41	43	43	41
28		32	40	44	45	63	52	62	62
35		42	46	54	62	75	62	66	68
42		49	49	61	63	69	67	72	79
56		59	61	67	69	82	77	82	83
90		71	70	77	80	83	81	86	87
7		1	2	3	0	3	1	5	4
14		8	8	8	3	7	7	7	12
21		9	8	12	12	16	22	13	26
28	20	13	12	17	11	20	23	20	24
35		12	12	20	17	22	24	21	27
42		13	14	29	23	29	32	25	40
56		28	17	37	30	34	40	37	52
84		53	33	53	40	57	62	46	61
7		0	0	0	0	0	1	0	0
14		0	4	0	3	0	3	4	5
21		0	0	9	0	2	9	2	11
28		4	7	1	2	6	13	4	20
35		0	2	9	3	7	17	9	14
42	10	3	3	6	6	6	20	9	14
56		8	1	18	10	14	24	18	13
70		14	5	16	12	19	24	16	26
84		15	9	22	13	20	36	24	40
98		14	15	32	18	22	24	27	21

*Means of duplicate treatments.

TABLE A-5. PERCENT LOSS* OF METHOXYCHLOR FROM NATURAL SEAWATER AS AFFECTED BY TEMPERATURE, SALINITY, AND STERILITY

Days incubation	Temp. °C	Salinity (ppt) 0 S	0 NS	10 S	10 NS	20 S	20 NS	28 S	28 NS
1		0	3.9	3.3	2.5	4.4	0	9.7	0
7		0	1.1	2.8	3.3	2.1	2.3	4.8	2.5
14		3.3	6.2	0	2.8	7.0	4.2	5.4	2.0
21		4.4	5.1	0	4.6	9.3	3.8	5.2	3.6
28	30	4.2	2.6	4.0	2.[illegible]	6.6	7.4	9.4	5.3
35		1.3	8.4	5.7	3.[illegible]	5.6	3.6	6.5	3.7
42		5.8	4.1	4.2	1.4	6.1	3.8	5.4	2.4
56		6.3	9.0	6.1	4.5	4.4	5.2	6.3	10.3
70		5.2	9.2	8.2	3.1	5.5	10.4	10.3	9.6
84		12.1	11.9	12.0	9.0	11.4	9.7	12.2	14.7
84	20	Largest losses observed at 20°C under sterile and nonsterile conditions were 8.5 and 5.1 percent, respectively.							
84	10	Largest losses observed at 10°C under sterile and nonsterile conditions were 1.1 and 1.2 percent, respectively.							

*Means of duplicate treatments.